

In the claims:

1. (Currently Amended) A method of identifying interactions between polypeptides comprising:
 - (a) expressing in a cell lacking Ras signaling:
 - (i) a first polynucleotide encoding a first bait polypeptide being capable of interacting with a plasmalemma of the cell and being operably linked to an inducible promoter; and
 - (ii) a second polynucleotide encoding a second prey polypeptide fused to a cytoplasmic Ras mutant, said cytoplasmic Ras mutant being capable of said Ras signaling if mobilized to said plasmalemma of said cell; and
 - (b) detecting restoration of said Ras signaling in said cell grown under:
 - (i) inductive conditions which result in expression of said first polypeptide from said inducible promoter; and
 - (ii) non-inductive conditions which result in substantially no expression of said first polypeptide from said inducible promoter,

wherein said a restoration of said Ras signaling present only in said cell grown under said inductive conditions is indicative of an interaction between said first polypeptide and said second polypeptide.

2. (Currently Amended) The method of claim 1, wherein said first bait polypeptide is a native transmembrane protein.

3-5. (Canceled)

6. (Previously Presented) The method of claim 1, wherein said cell lacking Ras signaling is a yeast cell exhibiting a mutant Ras phenotype characterized by growth suppression under non-permissive conditions.

7. (Original) The method of claim 6, wherein said cytoplasmic Ras mutant is capable of complementing said mutant Ras phenotype if mobilized to said plasmalemma of said cell.

8. (Previously Presented) The method of claim 1, wherein said first bait polypeptide includes an amino acid sequence for plasmalemma targeting.

9. (Currently Amended) A method of identifying interactions between polypeptides comprising:

(a) expressing in each cell of a plurality of cells lacking Ras signaling:

(i) a first polynucleotide encoding a first bait polypeptide being capable of interacting with a plasmalemma of said cells and operably linked to an inducible promoter; and

(ii) a second polynucleotide, said second polypeptide being of from a library of polynucleotides and wherein each polynucleotide of said library of polynucleotides encodes each encoding a distinct prey polypeptide fused to a cytoplasmic Ras mutant, said cytoplasmic Ras mutant being capable of said Ras signaling if mobilized to said plasmalemma of said cells; and

(b) identifying restoration of said Ras signaling in said cells grown under:

(i) inductive conditions which result in expression of said first polypeptide from said inducible promoter; and

(ii) non-inductive conditions which result in substantially no expression of said first polypeptide from said inducible promoter,

wherein said a restoration of said Ras signaling present only in said cells grown under said inductive conditions is indicative of an interaction between said first polypeptide and said distinct polypeptide.

10. (Currently Amended) The method of claim 9, further comprising isolating from each of said cells of said plurality of cells a polynucleotide encoding said distinct prey polypeptide.

11. (Currently Amended) The method of claim 9, wherein said first bait polypeptide is a native transmembrane protein.

12-14. (Canceled)

15. (Previously Presented) The method of claim 9, wherein said cells lacking said Ras signaling are yeast cells exhibiting a mutant Ras phenotype characterized by growth suppression under non-permissive conditions.

16. (Original) The method of claim 15, wherein said cytoplasmic Ras mutant is capable of complementing said mutant Ras phenotype if mobilized to said plasmalemma of said cells.

17. (Previously Presented) The method of claim 9, wherein said first bait polypeptide includes an amino acid sequence for plasmalemma targeting.

18. (Currently Amended) A method of identifying interactions between polypeptides comprising:

(a) expressing in each cell of a plurality of cells lacking Ras signaling:

(i) a first polynucleotide, said first polynucleotide being from of a library of polynucleotides and wherein each polynucleotide of said library of polynucleotides is operably linked to a first inducible promoter and each encoding encodes a first polypeptide being capable of interacting with a plasmalemma of said cells, said first polypeptide being fused to a second polypeptide; and

(ii) a second polynucleotide encoding a cytoplasmic Ras mutant fused to a third polypeptide and being operably linked to a

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second inducible promoter, said cytoplasmic Ras mutant being capable of said Ras signaling if mobilized to said-a plasmalemma of said cells, wherein said first inducible promoter and said second inducible promoter are induced by different inductive conditions; and

- (b) identifying ~~restoration~~ of said Ras signaling in said cells of said plurality of cells grown under:
 - (i) said different inductive conditions which result in expression of said first polynucleotide from said first inducible promoter and said second polynucleotide from said second inducible promoter; and
 - (ii) non-inductive conditions which result in substantially no expression of said first polynucleotide from said first inducible promoter and said second polynucleotide from said second inducible promoter,

wherein said-a restoration of said Ras signaling present only in said cells grown under said different inductive conditions is indicative of an interaction between said third polypeptide and said second polypeptide.

19. (Currently Amended) The method of claim 18, further comprising isolating from each of said cells of said plurality of cells a polynucleotide encoding said second polypeptide.

20. (Currently Amended) The method of claim 18, wherein said first polypeptide is a native transmembrane protein.

21-23. (Canceled)

24. (Previously Presented) The method of claim 18, wherein said cells lacking said Ras signaling are yeast cells exhibiting a mutant Ras phenotype characterized by growth suppression under non-permissive conditions.

25. (Original) The method of claim 24, wherein said cytoplasmic Ras mutant is capable of complementing said mutant Ras phenotype if mobilized to said plasmalemma of said cells.

26. (Original) The method of claim 18, wherein said first polypeptide includes an amino acid sequence for plasmalemma targeting.

27. (Currently Amended) A method of identifying interactions between polypeptides comprising:

(a) expressing in each cell of a plurality of cells lacking Ras signaling:

(i) a first polynucleotide, said first polynucleotide being from of a first library of polynucleotides, and wherein each polynucleotide of said first library of polynucleotides is operably linked to a first inducible promoter and encoding encodes a first polypeptide being capable of interacting with a plasmalemma of said cells, said first polypeptide being fused to a second polypeptide; and

(ii) a second polynucleotide, said second polynucleotide being from of a second library of polynucleotides, and wherein each polynucleotide of said second library of polynucleotides each is operably linked to a second inducible promoter and each encoding encodes a cytoplasmic Ras mutant fused to a third polypeptide, said cytoplasmic Ras mutant being capable of said Ras signaling if mobilized to said plasmalemma of said cells, wherein said first inducible promoter and said second inducible promoter are induced by different inductive conditions; and

(b) identifying restoration of said Ras signaling in said cells grown under:

(i) said different inductive conditions which result in expression of said first polynucleotide from said first inducible promoter and said second polynucleotide from said second inducible promoter; and

(ii) non-inductive conditions which result in substantially no expression of said first polynucleotide from said first inducible promoter and said second polynucleotide from said second inducible promoter,
wherein said-a restoration of said Ras signaling present only in said cells grown under said different inductive conditions is indicative of an interaction between said third polypeptide and said second polypeptide.

28. (Currently Amended) The method of claim 27, further comprising isolating from each of said cells of said plurality of cells polynucleotides encoding said second polypeptide and said third polynucleotides.

29. (Currently Amended) The method of claim 27, wherein said first polypeptide is a native transmembrane protein.

30-32. (Canceled)

33. (Previously Presented) The method of claim 27, wherein said cells lacking said Ras signaling are yeast cells exhibiting a mutant Ras phenotype characterized by growth suppression under non-permissive conditions.

34. (Original) The method of claim 33, wherein said cytoplasmic Ras mutant is capable of complementing said mutant Ras phenotype if mobilized to said plasmalemma of said cells.

35. (Original) The method of claim 27, wherein said first polypeptide includes an amino acid sequence for plasmalemma targeting.

36-49. (Canceled)

50. (Previously Presented) The method of claim 1, wherein said second polynucleotide is operably linked to a second inducible promoter and

whereas said first inducible promoter and said second inducible promoter are induced by different inductive conditions.

51. (Previously Presented) The method of claim 9, wherein said second polynucleotide is operably linked to a second inducible promoter and whereas said first inducible promoter and said second inducible promoter are induced by different inductive conditions.

52. (New) The method of claim 50, wherein said detecting said Ras signaling in said cell is further effected under:

- (i) said different inductive conditions which result in expression of said second polypeptide from said second inducible promoter ; and
- (ii) non-inductive conditions which result in substantially no expression of said second polypeptide from said second inducible promoter,

53. (New) The method of claim 51, wherein said detecting said Ras signaling in said cell is further effected under:

- (i) said different inductive conditions which result in expression of said second polypeptide from said second inducible promoter ; and
- (ii) non-inductive conditions which result in substantially no expression of said second polypeptide from said second inducible promoter,